Phytophthora Root Rot of Kiwifruit in Chile

B. A. LATORRE, Professor, and C. ALVAREZ, Research Assistant, Facultad de Agronomía, Pontificia Universidad Católica, Casilla 6177, Santiago, Chile; and O. K. RIBEIRO, Consultant Plant Pathologist and President, Microbiotica International, 10744 N. E. Manitou Beach Drive, Bainbridge Island, WA 98110

ABSTRACT

Latorre, B. A., Alvarez, C., and Ribeiro, O. K. 1991. Phytophthora root rot of kiwifruit in Chile. Plant Dis. 75:949-952.

Kiwifruit (*Actinidia chinensis*) has become a major fruit crop for exportation in Chile totalling about 30,000,000 t each year. A decline associated with a severe root rot has frequently appeared in 1- to 5-yr-old kiwifruit vines in the central zone of Chile. Our results demonstrate the presence of pathogenic isolates of *Phytophthora cryptogea* and *P. citrophthora* associated with diseased plants. This is the first report of these pathogens affecting kiwi in Chile. They are a potential threat for kiwi production, particularly in poorly drained soils.

Kiwifruit (*Actinidia chinensis* Planch.) was commercially introduced in Chile in 1978. At present, it is a major fruit crop for exportation with a net crop production of approximately 30,000,000 t annually (1). During the past 5 yr (1985–1990), numerous 1- to 5-yr-old kiwifruit vines throughout the kiwifruit-growing area of Chile have shown decline symptoms including leaf chlorosis, lack of shoot growth, leaf wilting, leaf scorch, and eventual plant collapse (Fig. 1). This syndrome is consistently associated with a rotting of the roots and less frequently with canker development at the crown. The cause was initially associated with root damage attributable to soil saturation or root asphyxiation. Further analysis demonstrated the presence of *Phytophthora* spp. on diseased roots (8). In this study, we report the etiology of this decline and root rot of kiwifruit vines in Chile.

Accepted for publication 19 March 1991.

MATERIALS AND METHODS

Isolation. Roots and crowns of kiwifruit with symptoms resembling typical *Phytophthora* infections were collected in 22 commercial orchards, transported in plastic bags to the laboratory, and held in a cold room at 0 C for 1–3 days before isolation. Selected pieces of rootlets taken from the margin of necrotic roots were rinsed for 10–15 min in tap water, and small segments (1–2 mm) were placed in petri dishes on a selective medium for the isolation of *Phytophthora*. The medium (CMA) contained (per liter) 17 g of cornmeal agar (Difco), 150 mg of ampicillin (Laboratorio Chile), 10 mg of piramicin (Delvocid, Gist-brocades, Holland), 16 mg of rifampicin (Rifaldin, Lepetit), 10 mg of benomyl (Benlate, E. I. du Pont de Nemours & Co., Wilmington, DE), 100 mg of PCNB (Brassicol, Hoescht), and 30 mg of hymexazol (Tachigaren, Sankyo Co. Ltd.) (11,14). Plates were incubated in the dark at 20 C, and emerging colonies were subcultured on CMA and stored on the same medium without hymexazol at 5 C.

Identification. Isolates were identified on the basis of colony morphology, mycelial characteristics, cardinal temperatures and production, morphology, and size of sporangia, oogonia, and antheridia (10,11,13,15).

Sporangial production was stimulated by flooding small segments (2–5 cm) of actively growing mycelium with carrot juice media (500 g of fresh carrots were boiled for 10–15 min in 1,000 ml of distilled water) at 20 C for 48 hr in plastic plates under continuous light. Two plates were flooded for each isolate tested. The mycelium was then rinsed with sterile distilled water and washed with a sterile mineral salt solution at 5 C. The latter solution contained (per liter) 2.36 g of $Ca(NO_3)_2$, 0.5 g of KNO_3, 1 g of $MgSO_4 \cdot 7H_2O$, and 1 ml of chelated iron solution (EDTA, 13 g/L; KOH, 7.5 g/L; and $FeSO_4 \cdot 7H_2O$, 24.9 g/L) (11). Plates were rinsed again with sterile distilled water and flooded with 1.5% soil solution for 48–72 hr at 20 C. Plates were examined daily for sporangia and those that were negative were treated again with salt solution.

Oospores were produced on V8 agar medium (11) amended with B-sitosterol (30 μg/ml), tryptophan (20 μg/ml),

thiamine (1 μg/ml), and $CaCl_2$ (100 μg/ml). *P. cinnamomi* Rands mating type A1 or A2 was placed on V8 agar plates 1 cm from the unknown isolate of *Phytophthora* to ascertain mating type. Each isolate was placed in three plates and incubated at 20 C for at least 21 days.

Cardinal temperatures. Each isolate was placed on three CMA plates and plates were incubated at 5, 24, 30, or 35 C. The radial growth of the mycelium was measured after 5 days of incubation. The experiment was repeated twice.

Inoculum. Inoculum was prepared from 8- to 10-day-old cultures growing on potato-dextrose agar (PDA). Cultures were suspended in sterile distilled water, ground for 15 min at high speed, and adjusted to about 10^6 mycelial fragments per milliliter with a hemacytometer.

Pathogenicity. Kiwifruit seedlings were grown in sterilized (122.5 g of methyl bromide + 2.5 g of chloropicrin for 72 hr at 25 C) soil mixture (2:1:1, organic soil/sand/loam soil). At the three- to four-leaf stage, plants (approximately 8–10 cm high) were pulled up, washed in tap water, and the roots were dipped for 15 min in the prepared inoculum suspension. Plants were then transferred to pots (14 × 15.5 cm) containing the same soil mixture but preinoculated with 20 ml of the inoculum suspension per plot. Three plants were inoculated per isolate, and an equal number of uninoculated plants were left as checks. Inoculated and uninoculated plants were randomly distributed on trays containing 1–2 cm of water to assure a constant flooding of the pots (6) and were left in a lathhouse for 20 days at 22–25 C. Three uninoculated plants, irrigated twice a day, also served as control. Pathogenicity was evaluated by the number of plants showing leaf chlorosis, necrosis, or a complete leaf collapse and by the percentage of roots with necrosis compared with the uninoculated controls. Roots from uninoculated plants that were not flooded allowed us to differentiate the effects of anaerobic conditions. The experiment was repeated twice.

Fruit of apple cv. Granny Smith, kiwi cv. Hayward, and orange cv. Thompson were surface-disinfested with 1% sodium hypochlorite for 2 min and inoculated with mycelium plug taken from actively growing mycelium on CMA. Each fruit was punctured with a 0.5-mm borer and the mycelium was placed in the hole. Four fruits were inoculated per isolate and an equal number were punctured and left as controls. Fruits were incubated for 8 days at 20 C in a moist chamber. The experiment was repeated three times.

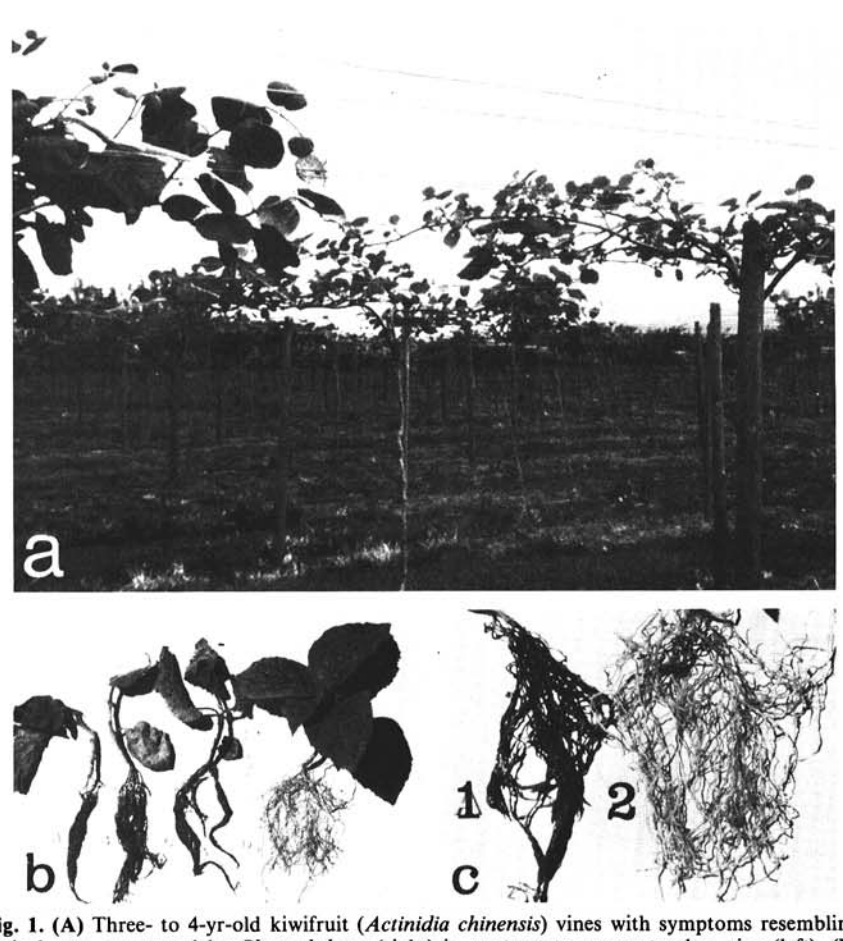

Fig. 1. (A) Three- to 4-yr-old kiwifruit (*Actinidia chinensis*) vines with symptoms resembling typical root rot caused by *Phytophthora* (right) in contrast to a symptomless vine (left). **(B)** Small seedlings inoculated with (from left to right) *P. cryptogea* isolates K-13-4 and K-15-2, *P. citrophthora* isolate K-14-1, and an uninoculated control. **(C)** Roots from plant inoculated with *P. cryptogea* isolate K-13-4 (left), maintained under continuous flooding for 20 days in comparison with uninoculated control (right) but flooded for the same time.

Table 1. Effect of temperature on radial growth of mycelium of *Phytophthora cryptogea* and *P. citrophthora* from kiwifruit

Isolate	Radial growth (cm)[a]			
	5 C	22 C	30 C	35 C
P. cryptogea				
K-13-4	T[b]	3.8	2.9	0.0
K-15-2	1.0	4.9	3.9	0.0
K-16-0	0.2	5.4	4.3	0.0
K-17-0	0.0	4.3	3.3	0.0
P. citrophthora				
K-14-1	0.5	3.7	5.4	0.0

[a] Radial growth on amended cornmeal agar after 5 days of incubation in the dark.
[b] T = trace growth.

RESULTS

Isolation and identification. *Phytophthora* spp. and *Pythium* spp. were isolated from five of 22 and 22 of 22 symptomatic kiwi roots, respectively. *Pythium middletonii* Sparrow (= *P. vexans* de Bary) was identified at the Commonwealth Agricultural Bureau International Mycological Institute (IMI), Kew, Surrey, England.

Based on morphology of sporangia and oospores and cardinal temperatures (Table 1), 14 isolates of *Phytophthora* were identified as *P. cryptogea* Pethybr. & Lafferty and two as *P. citrophthora* (R. E. Sm. & E. H. Sm.) Leonian.

Single cultures of *P. cryptogea* and *P. citrophthora* produced only sporangia on liquid medium. *P. cryptogea* formed ovoid to obpyriform, nonpapillate sporangia (Fig. 2A and B) with a mean length of 41 μm and a mean width of 27.9 μm. Internal proliferations and hyphal swellings (Fig. 2D) were observed on liquid carrot juice medium. Chlamydospores were not observed. Smooth-walled aplerotic oospores (average of 30 μm in diameter) were produced after pairing with A1 or A2 isolates of *P. cinnamomi*. Antheridia were amphigenous (Fig. 2C). *P. citrophthora* produced a radiate mycelium and papillate sporangia that were variable in shape and size, with one or more apices (Fig. 2E and F). Sporangial proliferation was not observed. Oospores were rarely formed when test cultures were paired with *P. cinnamomi* A1. Antheridia were

amphigenous (Fig. 2G). Hyphal swelling and chlamydospores were not observed.

Cardinal temperatures. All isolates of *P. cryptogea* and *P. citrophthora* did not grow at 35 C, and very little growth was recorded at 5 C (Table 1). Optimal growth was observed between 22 and 30 C.

Pathogenicity. *P. cryptogea* and *P. citrophthora* caused root decay on kiwi seedlings (Table 2). Inoculated plants developed chlorosis and wilting, and all but one inoculated plant died within 20 days after inoculation. Extensive decay of the entire root system was observed. Control plants (either flooded or nonflooded) showed no aerial symptoms, and their roots showed only a slight necrosis primarily at the tip of the feeder roots (Fig. 1B and C).

All isolates of *P. cryptogea* and *P. citrophthora* incited extensive decay on apple, kiwi, or orange fruits (Table 2). On apple, *P. cryptogea* produced an internal breakdown, and *P. citrophthora* mainly affected the cortical layers of the fruits.

DISCUSSION

Root rot and decline of kiwi has been described in California, Europe, New Zealand, and Australia (3,4,12,17). In California (3), it has been associated with *P. citrophthora*, *P. cryptogea*, and *P. megasperma* Drechs., whereas *P. cactorum* (Lebert & Cohn) J. Schröt., *P. cinnamomi*, *P. lateralis* Tucker & Milbrath, and *P. citricola* Sawada were found as causal agents of this syndrome elsewhere (12,17). In addition, an unidentified *Pythium* species was associated with root rot of kiwifruit in California (3). Our results indicated that *P. cryptogea* and *P. citrophthora* caused root rot of kiwifruit in the central zone of Chile. *P. cryptogea* was the species most frequently isolated.

The morphological characteristics (Fig. 1) and cardinal temperatures (Table 1) fit the descriptions given in the literature for *P. cryptogea* and *P. citrophthora* (10,11,13). These identifications were confirmed by the Commonwealth Agricultural Bureau IMI, Kew, Surrey, England. This is the first report of *P. cryptogea* in Chile. *P. citrophthora* was previously described on citrus trees in Chile (7,9).

The isolates of *P. cryptogea* from kiwifruit were found to be either mating type A1 or A2, and *P. citrophthora* was A2. These *Phytophthora* species are very aggressive and are a potential threat to kiwifruit production in Chile, particularly in poorly drained soils. As reported for this and other hosts, the disease is favored by abundant soil moisture (2,5,6,16).

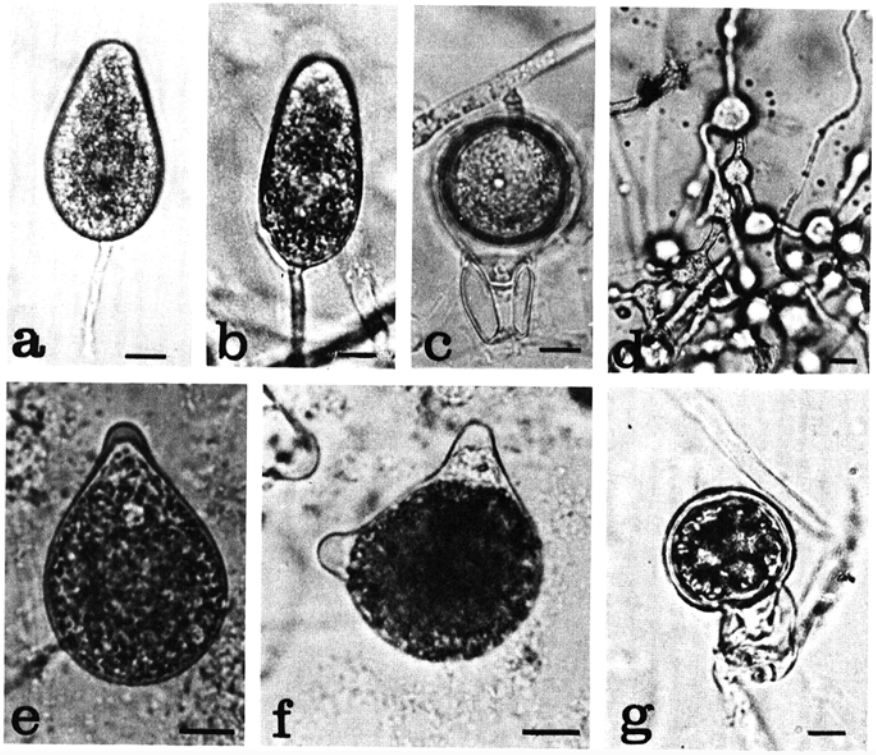

Fig. 2. **(A)** and **(B)** Ovoid to obpiriform papillate sporangia of *Phytophthora cryptogea*, **(C)** smooth-walled aplerotic oospore, with amphigenous antheridia, and **(D)** hyphal swellings. **(E)** Ovoid papillated sporangium of *P. citrophthora*, **(F)** ovoid papillated sporangium having two apices, and **(G)** smooth-walled aplerotic oospore, with amphigenous antheridia. Bars = 10 μm.

Table 2. Pathogenicity of *Phytophthora cryptogea* and *P. citrophthora* isolated from infected roots of kiwifruit in Chile

Isolate	Mating type	Root rot (%)[a]	Aerial collapse[b]	Fruit rot, diameter (cm)[c]		
				Apple	Kiwi	Orange
P. cryptogea						
K-13-4	A2	100	3	5.3	4.4	6.6
K-15-2	A2	100	2	5.8	4.0	6.9
K-17-0	A1	ND[d]	ND	5.1	5.1	6.3
K-16-0	A1	ND	ND	5.4	5.1	6.0
P. citrophthora						
K-14-1	A2	100	3	5.6	4.8	6.9
Uninoculated control[e]		0	0	0.0	0.0	0.0

[a] Relative to the root mass developed by the uninoculated, flooded controls.
[b] Number of affected plants out of three.
[c] Determined after 8 days of incubation at 20 C in a moist chamber on apple cv. Granny Smith, kiwi cv. Hayward, and orange cv. Thompson.
[d] ND = Not determined.
[e] Controls were three uninoculated plants left on trays containing 1–2 cm of water and three uninoculated plants irrigated twice a day.

ACKNOWLEDGMENTS

This research was supported in part by FONDECYT project 517-89. We thank E. Bartolloti and R. Vargas for their contribution to this study.

LITERATURE CITED

1. Anónimo. 1990. Estadísticas de exportaciones hortofrutícolas. Asociación de Exportadores de Chile A.G. Departmento de Computación y Estadísticas. Santiago, Chile. 98 pp.
2. Conn, K. E., and Gubler, W. D. 1989. Effect of flood duration on severity of Phytophthora root and crown rot of kiwifruit in California. (Abstr.) Phytopathology 79:1155.
3. Conn, K. E., Gubler, W. D., and Mircetich, S. M. 1988. *Phytophthora* spp. causing root and crown rot of kiwifruit in California. (Abstr.) Phytopathology 78:1573.
4. Farr, D. F., Bills, G. F., Chamuris, G. P., and Rossan, A. Y. 1989. Fungi on Plants and Plant Products in the United States. American Phytopathological Society, St. Paul, MN. 1252 pp.
5. Feld, S. J., Menge, J. A., and Stolzy, L. H. 1990. Influence of drop and furrow irrigation on Phytophthora root rot of citrus under field and greenhouse conditions. Plant Dis. 74:21-27.
6. Klotz, L. J., Stolzy, L. H., Letey, J., Labanauskas, C. K., Valoras, N., and DeWolfe, T. A. 1966. Root decay of citrus as affected by soil moisture and aeration. Calif. Citrogr. 51:296-299.
7. Latorre, B. A. 1989. Enfermedades de las Plantas Cultivadas. Segunda ed. Ediciones Universidad Católica, Santiago, Chile. 307 pp.
8. Morales, A., and Moreno, M. P. 1986. Kiwi: Pudriciones radiculares. Aconex 12:13-16.
9. Mujica, F. R., and Vergara, C. 1980. Flora Fungosa Chilena. Segunda ed. Ciencias Agrícolas 5, Facultad de Agronomía, Universidad de Chile, Santiago, Chile. 308 pp.
10. Newhook, F. J., Waterhouse, G. M., and Stamps, D. J. 1978. Tabular key to the species

of *Phytophthora* de Bary. Mycologial Papers 143. Commonw. Mycol. Inst./Assoc. Appl. Biol, Kew, Surrey, England. 20 pp.
11. Ribeiro, O. K. 1978. A Source Book of the Genus *Phytophthora*. J. Cramer, Lehre, Germany. 420 pp.
12. Sale, P. R. 1985. Kiwifruit Culture. Government Printing Office, Wellington, New Zealand. 96 pp.
13. Stamps, D. J. 1978. *Phytophthora cryptogea*. No. 592 in: Descriptions of Pathogenic Fungi and Bacteria. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England. 2 pp.
14. Tsao, P. H. 1970. Selective media for isolation of pathogenic fungi. Annu. Rev. Phytopathol. 8:157-186.
15. Waterhouse, G. M., Newhook, F. J., and Stamps, D. J. 1983. Present criteria for classification of *Phytophthora*. Pages 139-147 in: *Phytophthora*: Its Biology, Taxonomy, Ecology and Pathology. D. C. Erwin, S. Barniki-Garcia, and P. H. Tsao, eds. American Phytopathologial Society, St. Paul, MN.
16. Wilcox, W. F., and Mircetich, S. M. 1985. Effects of flooding duration on the development of Phytophthora root and crown rots of cherry. Phytopathology 75:1451-1455.
17. Youssef, S., and Bergamini, A. 1989. L'Actinidia sa Culture. La Maison Rustique, Paris, France. 63 pp.